PAIN RELIEF AGENTS

This application is a 371 of PCT/GB2003/004774, filed November 5, 2003 and claims priority from GB 0226105.5 filed November 8, 2002; the disclosure of which is incorporated herein by reference.

The present invention relates to pain relief agents and, in particular, pain relief agents which comprise one or more heat shock polypeptides.

Heat shock polypeptides are a family of molecules found in all organisms, whose function is to aid the biological processing and stability of biological molecules (Zugel & Kauffman (1999) *Role of heat shock polypeptides in protection from and pathogenesis of infectious diseases.* **Clin. Microbiol. Rev.** (12)1: 19-39; Ranford *et al.* (2000) *Chaperonins are cell signalling polypeptides: - the unfolding biology of molecular chaperones.* **Exp. Rev. Mol. Med.,** 15 September, www.ermn.cbcu.cam.ac.uk/00002015h).

Heat shock polypeptides are located in every cellular compartment, and possess the ability to interact with a wide range of biological molecules. In particular, the heat shock polypeptides aid and influence polypeptide folding and polypeptide translocation at any time from assembly through to disassembly of the polypeptide and any complexes thereof. The helper nature of the heat shock polypeptides has led to them to also being known as molecular chaperones (Laskey *et al.* (1978) *Nucleosomes are assembled by an acidic polypeptide, which binds histones and transfers them to DNA*. **Nature** (275): 416-420).

Heat shock polypeptides are synthesized by cells in response to environmental stress, which includes, but is not limited to temperature changes (both increases and decreases), and pathophysiological signals such as cytokines. In response to the environmental stress, heat shock

polypeptides use their ability to process other polypeptides to protect such polypeptides from any denaturation that may occur due to the presence of the stress. This mechanism also serves to protect cells which contain the protein.

Chaperonin polypeptides are a subgroup of heat shock polypeptides whose role in polypeptide folding is well known. There are two families of chaperonin polypeptide, the chaperonin 60 (approximately 60 kDa) and chaperonin 10 (approximately 10 kDa) families (Ranford, 2000). The best characterized chaperonins are those derived from *E. coli*, from which the characteristic structure of chaperonin 60 and chaperonin 10 has been established. The chaperonin complexes of most other organisms also substantially conform to this characteristic structure.

The characteristic structure of chaperonins is a complex formed from two heptamer rings (composed of seven chaperonin 60 monomers) which face one another and are capped by a heptamer ring composed of chaperonin 10 monomers.

Conventionally, chaperonins assist polypeptide folding when the target polypeptide enters the central core of the ringed heptamers, and on the subsequent release of energy from ATP the target polypeptide is released from the central core by a conformational change in the chaperonin structure (Ranson *et al.* (1998) *Review Article: Chaperones.* **Biochem. J** (333): 233-242).

Mycobacterium tuberculosis (*M. tuberculosis*) produces Chaperonin 60.1 (cpn 60.1), a polypeptide that is named based on its amino acid sequence identity to other known chaperonins. Further *M. tuberculosis* chaperonin

polypeptides are chaperonin 10 (cpn 10) and chaperonin 60.2 (cpn 60.2). Chaperonin 60.2 exhibits 59.6% amino acid sequence identity and 65.6% nucleic acid sequence identity to cpn 60.1.

Pain relief is usually achieved by oral or parenteral medication. Effective pain relief can be achieved in most cases with widely known pain relief drugs such as paracetamol, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) such as ibuprofen, and cylooxygenase-2-selective inhibitors (CSIs). Narcotic analgesics act on specific receptors in the Central Nervous System (CNS). Codeine and dihydrocodeine are moderately potent narcotic analgesics and have a low potential for addiction. Other more potent narcotic analgesics, such as morphine and methadone can be used to control severe pain.

A variety of problems exist with presently known pain relief agents. The drugs are relatively short acting and analgesia lasts for only a few hours. Repeated doses of the drug are usually necessary to control the pain. Sub-optimal pain relief is another common problem, leading to the patient increasing the dose, or changing medication. In the case of NSAIDS, unpleasant gastrointestinal side-effects such as dyspepsia and ulcers are common, and about two-thirds of users change brands of NSAIDS at least once because of adverse effects and poor efficacy (Steinfeld S and Bjorke PA. Results from a patient survey to assess gastrointestinal burden of non-steroidal anti-inflammatory drug therapy contrasted with a review of data from EVA to determine satisfaction with rofecoxib. **Rheumatology (Oxford)** 2002, 41(S1), 23-27.). In addition, NSAIDs and CSIs can give rise to cardiovascular complications (Hillis W S, (2000) Areas of emerging interest in analgesia: cardiovascular complications. **Am. J. Ther.** 9 (3) 259-69). Aspirin can cause Reye Syndrome in a small proportion of children,

and thus aspirin is not available for use in children. Paracetamol has to be used with caution since, an overdose, is hepatotoxic (Cranswick, N., Coghlan D. Paracetamol efficacy and safety in children: the first 40 years (2000) **Am. J. Ther.** 7(2) 135-41). Narcotic analgesics have a variety of side-effects including drowsiness, constipation, nausea, headache and vertigo. Repeated administration of potent narcotic analgesics such as morphine can cause addiction.

The present invention seeks to solve these problems in the following ways. An advantage of chaperonins as pain relief agents over current pain relief drugs is that they may have fewer adverse side-effects. It has been estimated that two billion people carry *M. tuberculosis* without developing Tuberculosis. Carriage of *M. tuberculosis* has not been associated with the side effects which are seen with commonly known pain-relief medication such as gastro-intestinal side-effects, cardiovascular complications, hepatotoxicity, Reye Syndrome or addiction.

A further advantage over previously known pain relief agents is that, the analgesic affect of chaperonins will last longer.

In a first aspect, the present invention provides the use of a heat shock polypeptide and/or its encoding nucleic acid sequence, in the manufacture of a medicament for use in the relief of pain.

Preferably the heat shock polypeptide is a chaperonin. More preferably the chaperonin is derived from a bacterium. Yet more preferably the chaperonin is derived from Mycobacterium. Most preferably the chaperonin is derived from *Mycobacterium tuberculosis*.

Preferably the nucleic acid comprises:

(i) the nucleotide sequence of Figure 1 (SEQ ID NO:1) and/or Figure 2 (SEQ ID NO:3) and/or Figure 3 (SEQ ID NO:5), or

(ii) a sequence which has more than 66% identity to sequence (i), or a sequence which hybridizes to sequence (i) under conditions of 2 x SSC, 65°C (wherein SCC = 0.15M NaCl, 0.15M sodium citrate, pH 7.2) which encodes a functionally equivalent polypeptide to the sequence encoded by the nucleotide sequence of Figure 1 (SEQ ID NO:1) and/or Figure 2 (SEEQ ID NO:3) and/or Figure 3 (SEQ ID NO:5), or

(iii) a fragment of sequence (i) or (ii) encoding a functionally equivalent polypeptide fragment.

Preferably the heat shock polypeptide comprises:

(i) the amino acid sequence of Figure 1 (SEQ ID NO:2) and/or Figure 2 (SEQ ID NO:4) and/or Figure 3 (SEQ ID No:6), or

(ii) a sequence which has more than 60% identity to sequence (i) which provides a functionally equivalent polypeptide, or

(iii) a functionally equivalent fragment of sequence (i) or (ii).

Preferably the functionally equivalent fragments are from 3 to 400 residues in length. Yet more preferably the functionally equivalent fragments are from 3 to 100 residues in length.

Preferably the nucleic acid molecule encodes a functionally equivalent fragment as defined above.

Preferably the medicament further comprises a pharmaceutically acceptable excipent, diluent or carrier.

More preferably the medicament is provided in combination with at least one additive for assisting or augmenting the action of the nucleic acid molecules or polypeptides.

Yet more preferably the additive is selected from at least one of paracetamol, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) such as ibuprofen, and cylooxygenase-2-selective inhibitors (CSIs), opiates, such as morphine and heroin.

Preferably the medicament provides prolonged or sustained relief.

Preferably the daily dosage level of will be from 0.0001 to 100,000 mg, administered in single or divided doses. More preferably the daily dosage level is 0.0001 to 1000 mg.

In a preferred embodiment the time between dose administrations to the patient is between six and twelve hours.

Preferably the time between dose administrations to the patient is between nine and twelve hours after the previous dose.

In a further embodiment the time between dose administrations to the patient is between 12 days to 6 months. In a yet further preferred embodiment the time between dose administrations is between 12 hours to 12 days.

Preferably the compositions of the invention are formulated to permit administration by at least one selected from the intranasal, oral, parenteral, topical, ophthalmic, suppository, pessary or inhalation routes.

More preferably the compositions of the invention are formulated to permit administration by inhalation.

Preferably the medicament is used in pain relief of a human or animal patient. Most preferably the patient is a human.

In a second aspect, the present invention additionally provides a method comprising administering to a patient an amount of a medicament for the relief of pain, as described according to the first aspect of the invention.

Definitions

By "use in the relief of pain" we include any treatment which influences the pain felt by an individual, such influence including a delay in the onset, a reduction in the severity, a reduction of the duration, and/or the removal of the feeling of pain.

By "additive" we mean an ingredient that is provided in addition to the main medicament that is pharmacologically active either independently or in combination with the main medicament, whereby its presence in the medicament assists or augments the action of the main medicament.

By "hyperalgesia" we mean an earlier onset, an increase in the severity, an increase of the duration, and/or increased susceptibility to the feeling of pain.

By "functionally equivalent" we mean polypeptides and polypeptide fragments, which possess a pain relieving activity. This activity is preferably substantially the same or more preferably greater than the pain relieving activity of chaperonins derived from *Mycobacterium tuberculosis*. Functional equivalence can be measured using the methods as described in the examples e.g. Paw latency on a heated plate.

By "polypeptide" we also include peptides, proteins and peptidomimetic compounds. The term "peptidomimetic" refers to a compound that mimics the conformation and desirable features of a particular peptide as a therapeutic agent, but that avoids the undesirable features.

By "identity" we mean the number or percentage (dependent on presentation of the results) of nucleic acid residues in a candidate sequence that are identical with the nucleic acid residues of the sequence of interest, after aligning the sequences and introducing gaps, if necessary to achieve maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.[[.]] The percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned optimally.

The alignment may alternatively be carried out using the Clustal W program (Thompson *et al.*, (1994) *Nucleic Acids Res.* **22**, 4673-80). The parameters used may be as follows:

Fast pairwise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.

Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.
Scoring matrix: BLOSUM.

Preferred Embodiments

Examples embodying certain preferred aspects of the invention will now be described with reference to the following figures in which:-

PWL = Paw withdrawal latency
PWD = Paw withdrawal duration
VFF 4.31 = Von Frey monofilament – 4.31 calibre
VFF 5.07 = Von Frey monofilament – 5.07 calibre

Figure 1 – Amino acid (SEQ ID NO:2) and nucleic acid (SEQ ID NO:1) sequences of *Mycobacterium tuberculosis* Chaperonin 60.1.

Figure 2 – Amino acid (SEQ ID NO:4) and nucleic acid (SEQ ID NO:3) sequences of *Mycobacterium tuberculosis* Chaperonin 60.2.

Figure 3 – Amino acid (SEQ ID NO:6) and nucleic acid (SEQ ID No:3) sequences of *Mycobacterium tuberculosis* Chaperonin 10.

Figure 4 – Vomes Fry testing of cpn 60.1. Shows the number of paw withdrawals per 10 trials with two different Von Frey monofilaments (4.31 and 5.07) in the presence and absence of Mt cpn 60.1.

Figure 5 – PWL/PWD testing of cpn 60.1. Shows the duration of the responses of paw withdrawal in animals on the hot plate (upper panel) and on the cold plate (lower panel).

Figure 6 – Vomes Fry testing of Cpn 60.2. Shows the number of paw withdrawals per 10 trials with two different Von Frey microfilament calibers in the presence and absence of Mt cpn 60.1.

Figure 7 – PWL/PWD testing of Cpn 60.2. Shows the duration of the responses of paw withdrawal in animals on the hot plate (upper panel) and on the cold plate (lower panel).

Figure 8 – Vomes Fry testing of cpn 10. Shows the number of paw withdrawals per 10 trials with two different Von Frey monofilaments in the presence and absence of Mtcpn10.

Figure 9 – PWL/PWD testing of cpn 10. Shows the duration of the responses of paw withdrawal in animals on the hot plate (upper panel) and on the cold plate (lower panel).

Example 1 - Experimental testing of heat shock polypeptides in vivo

Experimental testing of heat shock polypeptides was investigated in test animals, separated into groups. Certain groups had induced hyperalgesia (i.e. an increased sensitivity to pain) and the effects of heat shock

polypeptides on normal and hyperalgesic animals was observed and measured.

Methods and Materials

The analgesic effect of chaperonins can be measured using the model for inflammatory pain described in Kanaan *et al.* (1996) *Pain* 66, p373-379, the disclosure of which is incorporated herein by reference. This model is based on endotoxin (ET)-induced inflammatory hyperalgesia in rats and mice.

A brief description of the methods employed are presented below.

Adult (200-250g) male Sprague-Dawley rats and adult (20-30g) male Balb/c mice were used. The animals were separated into four groups:

Group 1 – No injection.
Group 2 – Endotoxin only.
Group 3 – Endotoxin and Heat shock polypeptide.
Group 4 – Heat shock polypeptide only.

Injection of test substrates

Groups 2 and 3 were injected subcutaneously into the left hind paw with 1.25Tg ET prepared from *Salmonella typhosa,* 0901 (Difco, Detroit, Michigan, USA). Groups 1 and 4 received no endotoxin but instead received sterile physiological saline injected in the same manner. Groups 3 and 4 additionally received 1 Tg/ip of heat shock polypeptide injected in the same manner but not same mixture.

Behavioral observation

After injection, each animal was observed 48 hours.

Temperature plate test

The animals were individually placed on a hot surface plate in which the temperature was adjusted between 52.8 and 53.3°C, or a cold surface plate in which the temperature was adjusted between 4.8 and 5.3°C. The latency of the first sign of paw licking or jumping to avoid heating pain was taken as an index of the pain threshold.

Von Frey monofilament testing for mechanical allodynia

The method of Von Frey testing is disclosed in El-Khoury C *et al.* Neuroscience 2002, 112: 541-553 as incorporated herein.

Briefly, rats are placed in individual compartments of an elevated cage with a floor made of wire grid. The plantar surface of the hind paws is stimulated by Von Frey monofilaments (VFF) with increasing force. Two different monofilaments are used of different calibers (VFF 4.31(lowest) and VFF 5.07(highest)) in the range of 15-18.5 mN and 100-110mN respectively. Paw withdrawals per 10 trials are recorded.

Experimental protocols and data analysis

To determine the effects of an ET and/or heat shock polypeptide, a set (n=5) of animals with representatives of each group (1 to 4) were subjected to the

pain test for 3 consecutive days. Each animal was subjected to pain tests at the time intervals of 3, 6, 9 and 24 hours after the ET injection.

The degree of significance of variations between control and experimental values for each pain test was assessed by ANOVA test.

Heat shock polypeptides tested

The preferred methods were tested with the *Mycobacterium tuberculosis* chaperonin polypeptides, cpn 60.1, cpn 60.2 and cpn 10. Synthesis of these proteins can be achieved by using the sequences encoding the polypeptide constituting the compound of the invention as disclosed herein in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23 August 1988 to Goeddel *et al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

Results

The results are shown in figures 4 to 9 and it is clear that all three of the chaperonins tested exhibit a strong analgesic effect.

Example 2: analgesic effect of cpn 60.1

Figure 4 shows the number of paw withdrawals per 10 trials with two different Von Frey monofilaments (4.31 and 5.07) in the presence and absence of *M. tuberculosis* cpn 60.1. Both tests gave the same broad pattern of results.

In track one of each time point, the negative control had a background of approximately one PWD. In track two, the positive control (injected ET or lipopolysacccharide) increased to a maximum of about 6 (VFF 4.31) and 9.5(VFF 5.07) PWD. Track three, demonstrates the effect of cpn 60.1 treatment of ET induced hyperalgesia, and shows a general reduction to background levels in PWD at time points 3-9 hours. Track 4 shows the effect of cpn60.1 injected on its own, i.e. that there is no difference over that seen in the non-injected control group.

These results demonstrate that cpn60.1 reduces the hyperalgesia which is induced by endotoxin.

Figure 5 shows the duration of the responses of paw withdrawal in animals on the hot plate (PWL/Heat) and on the cold plate (PWD/Cold). The hot plate results show that there is no difference between the different time points and treatments, except for ET treated animals at time points 3-9 hours when the duration of latency is reduced to 4-5 seconds. The cold plate results show that none of the PWD are above the background with the exception of endotoxin injection, which shows a raised PWD at time points 3-9 hours. These results indicate that cpn 60.1 reduces hyperalgesia which is induced by endotoxin.

Example 3: analgesic effect of cpn 60.2

Figure 6 shows the number of paw withdrawals per 10 trials with two different Von Frey microfilament calibers (VFF 4.31 and VFF 5.07) in the presence and absence of *M. tuberculosis* cpn 60.2. Both tests broadly give the same pattern of results.

In track one of each time point, the negative control had a background of approximately less than one PWD in VFF4.31 and less than 2.5 PWD in VFF 5.07. In track two, the positive control (injected ET or lipopolysacccharide) increased to a maximum of about 6 (VFF4.31) and 7.5 (VFF5.07) PWD. In track three, the effect of cpn 60.2 treatment on ET induced hyperalgesia is shown, this demonstrates a reduction to background levels (control levels) in PWD at time points 3-9 hours. Track 4 shows the effect of cpn 60.2 injected on its own such that there is no effect over that seen in the non-injected control group. These results demonstrate that cpn 60.2 reduces the hyperalgesia which is induced by endotoxin.

Figure 7 shows the duration of the responses of paw withdrawal in animals on the hot plate (PWL/Heat) and on the cold plate (PWD/Cold). The hot plate results show that there is no difference between the different time points and treatments, except for ET treated animals at time points 3 and 6 hours when the duration of latency is reduced to 4-5 seconds. The cold plate results show that none of the PWD are above the background with the exception of ET which is considerably raised at time points 3-9 hours. These results indicate that this cpn 60.2 reduces hyperalgesia which is induced by endotoxin.

Example 4: analgesic effect of cpn 10

Figure 8 shows the number of paw withdrawals per 10 trials with two different Von Frey monofilaments (VFF 4.31 and VFF 5.07) in the presence and absence of *M. tuberculosis* cpn 10. Both tests broadly give the same pattern of results.

In track one of each time point, the negative control had a background of approximately less than one PWD. In track two, the positive control (injected ET or lipopolysacccharide) increased to a maximum of about 6 (VFF 4.31) and 8 (VFF 5.07) PWD. In track three, the effect of cpn 10 treatment of ET induced hyperalgesia on PWD is shown, for VFF 4.31 there is a reduction to background levels in PWD at time point 3 hours and just above background at time points 6 and 9 hours. For VFF 5.07 cpn 10 shows a smaller reduction, to 3.5 at 3 hours, to 3 at 6 hours, to 2.5 at 9 hours and no reduction at 24 hours. Track 4 shows the effects of cpn 10 injected on its own which demonstrates no effect over that seen in the non-injected group, except for VFF 4.31 where cpn 10 induced approximately one PWD at the 3 hour time point. These results demonstrate that cpn 10 reduces the hyperalgesia which is induced by ET.

Figure 9 shows the duration of the responses of paw withdrawal in animals on the hot plate (PWL/Heat) and on the cold plate (PWD/Cold). The heat plat test shows that there is no marked difference between the different time points and treatments, except for ET treated animals at time points 3-9 hours when the duration is reduced to 4-5 seconds. The cold plate test shows that none of the PWD are above the background with the exception of ET and ET in combination with cpn 10 which is considerably raised at time points 3-9 hours. At these time points cpn 10 still reduced the duration by about 50%.

Hence, cpn 10 is effective at reducing endotoxin induced hyperalgesia.

Example 5: Pharmaceutical compositions

A further aspect of the invention provides a pharmaceutical formulation comprising a heat shock polypeptide (the medicament) in admixture with a pharmaceutically or veterinarily acceptable adjuvant, diluent or carrier that is selected with regard to the intended route of administration and standard pharmaceutical practice. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free.

The formulations may conveniently be presented in unit dosage form containing a daily dose or unit or an appropriate fraction thereof, of the medicament and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the medicament with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the medicament with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compounds of the invention can be administered orally or by any parenteral route, in the form of a pharmaceutical formulation comprising the medicament, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form.

Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

The compounds of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the compounds of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The compounds of invention may also be administered *via* intracavernosal injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC), sucrose, gelatine and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatine capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or

dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intra-muscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention will usually be from 0.0001 to 100,000 mg per adult, administered in single or divided doses.

Thus, for example, the tablets or capsules of the compound of the invention may contain from 0.0001 mg to 100,000 mg of active compound for administration singly or two or more at a time, as appropriate. It is envisaged that a 500 mg tablet or capsule would be appropriate for single, repeat doses of one or more tablets or capsules. The physician in any event will determine the actual dosage, which will be most suitable for each individual patient, and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

The compounds of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container, pump, spray or nebulizer with the use of a suitable propellant, *e.g.* dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoro-ethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container, pump, spray or nebulizer may contain a solution or suspension of the active compound, *e.g.* using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, *e.g.* sorbitan trioleate. Capsules and cartridges (made, for example, from gelatine) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains between 0.001mg and 2g of a compound of the invention for delivery to the patient. It will be appreciated that he overall daily dose with an aerosol will vary from patient to patient, and may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the compounds of the invention can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, ointment or dusting powder. The compounds of the invention may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular route, particularly for treating diseases of the eye.

For ophthalmic use, the compounds of the invention can be formulated as micronized suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds of the invention can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid

paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Formulations suitable for topical administration in the mouth include lozenges comprising the medicament in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the medicament in an inert basis such as gelatine and glycerin, or sucrose and acacia; and mouth-washes comprising the medicament in a suitable liquid carrier.

Generally, in humans, oral, topical or inhalation administration of the compounds of the invention is preferred, being the most convenient. In circumstances where the recipient suffers from a swallowing disorder or from impairment of drug absorption after oral administration, the drug may be administered parenterally, *e.g.* sublingually or buccally.

For veterinary use, a compound of the invention is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration that will be most appropriate for a particular animal.

Example 6: Methods of pain relief

The compounds of the invention will provide effective pain relief in the following incidences of pain: backache, headache, toothache, earache, Arthritis, Gout, soft tissue trauma, ligament/tendon traumatic damage, broken bones, cancer, post operative pain, menstrual pain, obstetric pain, renal tract pain, visceral pain, burns, abscesses and other infections.

The suggested treatment route and regimen for the treatment of any of these conditions is the administration of 0.1mg to 1 gram once every 12 hours by inhalation delivered via an inhaler. However the skilled person would know that the most appropriate treatment regime would be dependent on the individual and the severity of the pain being felt.